

## Supporting Information

# A bifunctional non-natural tetrapeptide modulates amyloid-beta peptide aggregation in the presence of Cu(II)

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### Experimental Section

#### Materials and Procedures

**Synthesis and purification of A $\beta$ (1-40), DP and TP peptides.** The A $\beta$ (1-40) (NH<sub>2</sub>-DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV-NH<sub>2</sub>) peptide was synthesized using standard solid phase Fmoc chemistry in presence of 10% Anisole<sup>1</sup>, using Fmoc-Rink amide AM resin. Synthetic A $\beta$ (1-40) peptide was obtained as a trifluoroacetic acid (TFA) salt. Synthetic A $\beta$ (1-40) crude peptide was dissolved in 1% NH<sub>4</sub>OH in Milli-Q water (Millipore Corp., Milford, MA) at a concentration of 1.0–2.0 mg/mL<sup>2,3</sup>, pH >10, and centrifuged for 20 min at 14,500 rpm, and the supernatant was injected into a Superdex 75 preparative on an AKTA FPLC (GE Healthcare; Piscataway, NJ) system. Peptide was eluted in isocratic mode at a flow rate of 0.3 mL/min, using a 1% NH<sub>4</sub>OH (pH > 10) solution as a mobile phase. Peptide purity was determined by analytical reversed phase (RP) HPLC and was found to be > 98%. The molecular weight of A $\beta$ (1-40) was determined by electrospray ionization mass spectrometry (ESI-MS). DP (NH<sub>2</sub>-D-Trp-Aib-NH<sub>2</sub>) and TP (NH<sub>2</sub>-Met-Asp-D-Trp-Aib-NH<sub>2</sub>) peptides were synthesized using solid phase Fmoc chemistry, purified by high pressure liquid chromatography (HPLC) using a C18 Waters microBondapak column, and characterized by electro-spray mass spectrometry (ES-MS). The chromatograms of TP and DP show retention times of 18 min and 3 min, respectively. ES-MS analysis of the purified peptides showed their exact mass: 4328.82 for A $\beta$ (1-40), 288.26 for DP and 534.23 Da for TP. The DP and TP lyophilized peptides were stored in a desiccator at room temperature, while A $\beta$ (1-40) was stored in a desiccator at 4–8 °C.

**Sample preparation.** A $\beta$ (1-40) peptide was solubilized using the Alkali treatment<sup>3</sup>. Briefly, lyophilized A $\beta$ (1-40) was dissolved in 400 mM NaOH solution to a final peptide concentration of 1 mg/mL (231  $\mu$ M), ensuring that the pH of the resulting solution was  $\geq$  10.9. Solution was sonicated (3 cycles of 5 min each with 2 min rest) in a water bath sonicator and centrifuged for 10 min at 14,500 rpm. Supernatant was filtered through a PVDF membrane of 0.1  $\mu$ m pore and quantified by electronic absorption spectroscopy ( $\epsilon_{280\text{nm}} = 1,490 \text{ M}^{-1} \text{ cm}^{-1}$ ). Stock A $\beta$ (1-40) solutions were prepared fresh for each experiment. The DP and TP lyophilized peptides were dissolved in water, filtered with a PVDF membrane of 0.1  $\mu$ m pore and quantified by electronic absorption ( $\epsilon_{280\text{nm}} = 3.33 \text{ mM}^{-1} \text{ cm}^{-1}$  and  $\epsilon_{280\text{nm}} = 3.77 \text{ mM}^{-1} \text{ cm}^{-1}$ , respectively). DP solutions and TP were stored under refrigeration (4–8 °C). From these stock solutions, A $\beta$ (1-40) (20  $\mu$ M) or TP and DP (40  $\mu$ M) solutions were prepared by dilution into buffer (20 mM NEM, 10 mM NaCl and pH = 7.4). In the presence of copper, the concentration used was 0.4 equivalents of CuCl<sub>2</sub>.

**Electronic circular dichroism (CD) spectroscopy.** A solution of A $\beta$ (1-16) (0.3 mM) with Cu(II) at pH 7.4 was titrated with TP, and monitored by CD; subsequent additions of 0.12 equiv of TP (up to 1.5 equiv) were added. Also, a titration of TP (0.3 mM) by Cu(II) was performed by sequential additions of 0.2 equiv (up to 1 equiv) of Cu(II). In both cases, the circular dichroism spectra in the UV-Visible region were collected from 300 to 800 nm (every 2 nm) with a scan speed of 100 nm/min and a bandwidth of 5 nm. Spectrosil grade quartz cells with 1 cm path length and a Jasco J-815 spectropolarimeter were used.

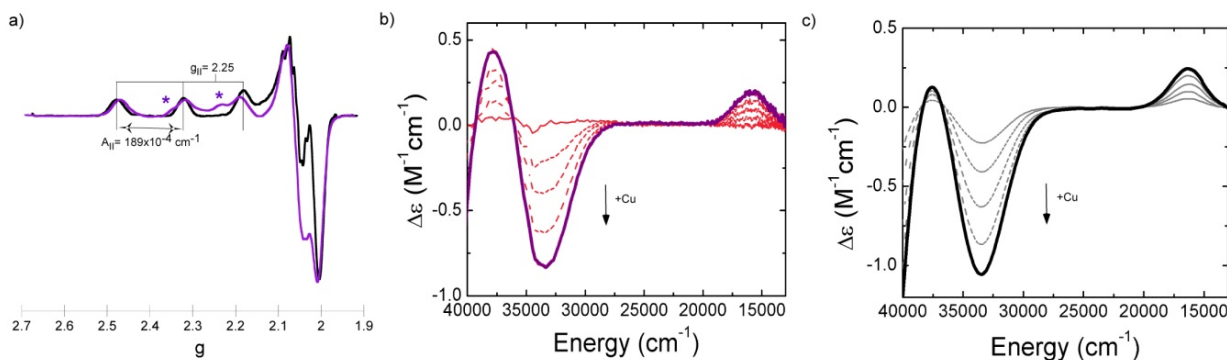
**Electron paramagnetic resonance (EPR) spectroscopy.** EPR spectra of the following samples were collected: MDVFMK and TP (both 0.3 mM with 1 equiv of Cu(II)), A $\beta$ (1-16) with Cu(II) and the final sample after titration of A $\beta$ (1-16)+Cu(II) by TP. Samples were placed in 4 mm quartz tubes. For EPR analysis of aggregates, in order to obtain samples with a concentration of Cu(II) high enough to be detected by EPR, aggregates from the end point of three ThT kinetic traces were pooled and resuspended into 250  $\mu$ L of buffer before loading into the quartz cell. X-band EPR spectra were collected using an EMX Plus Bruker System, with an ER 041 XG microwave bridge and an ER 4102ST cavity. The following conditions were used: microwave power, 10 mW; modulation amplitude, 8 G; modulation frequency, 100 kHz; time constant, 327 ms; conversion time, 82 ms; and averaging over 12 scans. EPR spectra were recorded at 150K, using a ER4131VT variable temperature nitrogen system.

**A $\beta$ (1-40) aggregation assays monitored with ThT fluorescence spectroscopy.** The A $\beta$ (1-40) peptide was diluted (final concentration 20  $\mu$ M) in buffer (20 mM NEM, 10 mM NaCl and pH= 7.4; previously filtered) with ThT (20  $\mu$ M). The fluorescence emission spectra from 460 to 550 nm with excitation  $\lambda$ = 450 nm were collected as a function of time after dilution of A $\beta$ (1-40) into the aqueous buffer. Assays in the presence of DP and TP were performed at 40  $\mu$ M. Experiments were performed at 37 ° C and constant agitation, using plastic cells (with a 1 cm path length) and a Varian Cary Eclipse fluorometer.

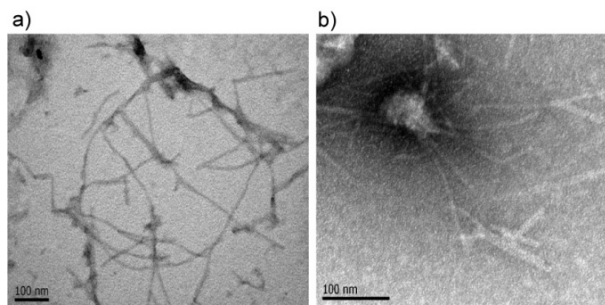
**Monitoring the particle size of A $\beta$ 1-40 at early times of aggregation by dynamic light scattering (DLS).** The A $\beta$ (1-40) peptide was diluted (final concentration 20  $\mu$ M) in buffer (20 mM NEM, 10 mM NaCl and pH= 7.4, previously filtered). DP and TP were used in a concentration of 40  $\mu$ M. Experiments were performed at 37 ° C, without stirring, monitoring each 10 min and using spectroil grade quartz cells (with a 1 cm path length) and a ZS90 Zetasizer Nano series spectrometer (Malvern).

**Transmission electron microscopy (TEM).** A $\beta$ (1-40) fibers grown in the aggregation assays monitored by ThT were placed on copper grids for 2 min, the grids were dried with filter paper and stained with uranyl acetate 2% (two cycles of 30 sec incubations followed by drying for 2 min). The grids were observed using a JEOL-JEM 1400 EX transmission electron microscope. The same procedure was performed with the samples taken at early times of the aggregation assay (t=0 and 40 min). These samples were simultaneously analyzed by DLS, ThT fluorescence and TEM.

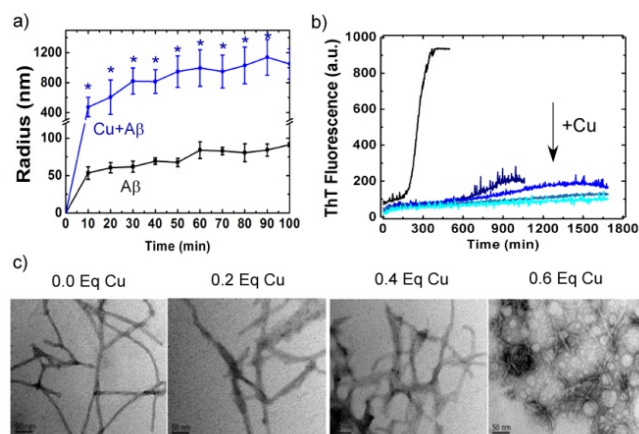
**Statistical Analysis.** DLS and ThT fluorescence data were analyzed by one-way ANOVA post-hoc Tukey. The area under the curve (AUC) was also analyzed by ANOVA. DLS and ThT fluorescence data are expressed as the mean  $\pm$  standard error of at least triplicate experiments (for A $\beta$  n=9, A $\beta$ +DP n=8, A $\beta$ +TP n=6, A $\beta$ +Cu n=7, A $\beta$ +DP+Cu n=7 and A $\beta$ +TP+Cu n=3).



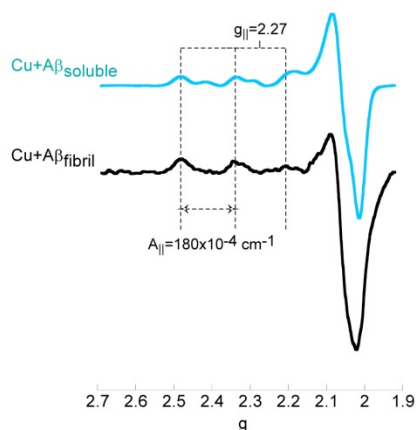
**Fig. S1 TP binds Cu(II) in a similar fashion as MDVFMK.** a) Comparison of EPR spectra of MDVFMK (black line) and TP (purple line) 0.3 mM with one equivalent of Cu(II); \* symbols denote signals associated to a small amount of free Cu(II) in solution. b) Titration of TP 0.3 mM (solid red line) by Cu(II), as followed by CD; sequential additions of 0.2 equiv of Cu(II) (dash red lines) were made up to 1 equiv (purple line). c) Titration of MDVFMK at 0.3 mM by Cu(II), as followed by CD; sequential additions of 0.2 equiv of Cu(II) (dash gray lines) were made up to 1 equiv (black line). These data clearly show that TP, containing residues Met-Asp is capable of chelating Cu(II) in a similar fashion as the first six aminoacids of  $\alpha$ -synuclein, MDVFMK, which constitute the high affinity ( $K_d = 0.2 \mu$ M) binding site for Cu(II) in this protein.



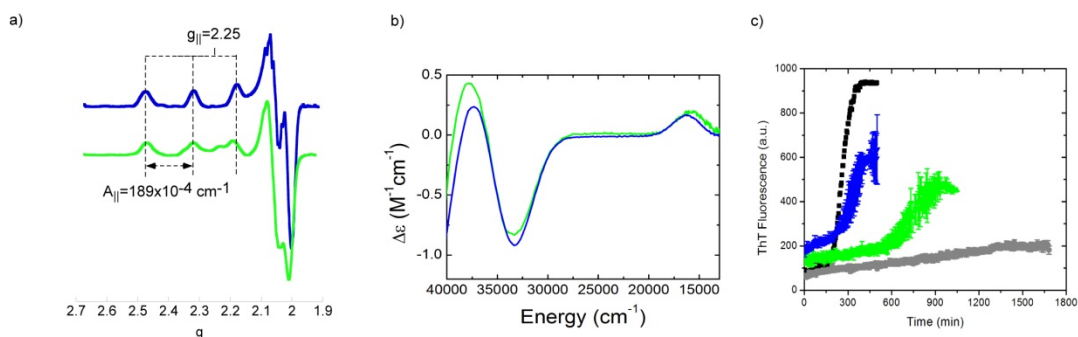
**Fig. S2 Effect of DP in A $\beta$ (1-40) aggregation.** TEM images of the final A $\beta$ (1-40) aggregates in the presence of DP (a) and in the presence of 0.4 equiv of Cu(II) and DP (b). Samples were collected at the end point of the ThT fluorescence assays shown in figures 2b and 3b.



**Fig. S3 Effect of Cu(II) in A $\beta$ (1-40) aggregation.** a) Particle hydrodynamic radius determined by DLS as a function of time after mixing A $\beta$ (1-40) 20  $\mu$ M into aqueous buffer, in the absence (black line) and presence (blue line) of 0.4 eq Cu(II); blue asterisks indicate that an accurate measurement of the hydrodynamic radius could not be achieved because the particle radius is much greater than 500 nm. b) A $\beta$ (1-40) fibril growth detected by ThT fluorescence at 0, 0.2, 0.4, 0.6 and 1 equiv of Cu. c) TEM images of the final aggregates formed in the presence of 0, 0.2, 0.4 and 0.6 equiv of Cu(II); samples were collected at the end points of the traces shown in b).



**Fig. S4 EPR analysis of Cu(II) in A $\beta$ (1-40) aggregates.** EPR spectrum of A $\beta$ (1-40) aggregates collected at the end point of aggregation assays in the presence of 0.4 equiv of Cu(II) is shown in black. For comparison, the EPR spectrum of Cu(II) bound to soluble A $\beta$ (1-16) is shown in cyan.



**Fig. S5 MDV chelates Cu(II) but it does not modulate A $\beta$ (1-40) aggregation.** a) EPR spectra of the Cu(II) complexes with TP (green line) and MDV (blue line) 0.3 mM with 1 equiv of Cu(II). b) CD spectra of the Cu(II) complexes with TP (green line) and MDV (blue line), same concentrations as in a). Data shown in a) and b) clearly show that the peptide MDV is capable of chelating Cu(II) in the same manner as TP, given that it contains the minimal construct Met-Asp for Cu(II) chelation. c) A $\beta$ (1-40) (20  $\mu$ M) fibril growth kinetics detected by ThT fluorescence in the absence (black line) and presence of 0.4 equiv of Cu(II) (gray line), and in the presence of both, TP (40  $\mu$ M) and 0.4 equiv of Cu(II) (green line); and in the presence of both, MDV (40  $\mu$ M) and 0.4 equiv of Cu(II) (blue line). The traces shown in c) clearly show that MDV recovers the sigmoidal behavior of A $\beta$ (1-40) aggregation in the absence of copper, consistent with it chelating

Cu(II) with high affinity. However, MDV does not have an effect on the lag time (nucleation phase), as TP does (green trace), consistent with MDV lacking residues that would confer it  $\beta$ -breaker properties.

#### References.

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